

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Lorin R. DeBonte, et al.                      Art Unit : Unknown  
Serial No. :    Examiner : Unknown  
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Title : FATTY ACID DESATURASES AND MUTANT SEQUENCES THEREOF

Commissioner for Patents  
Washington, D.C. 20231

**PRELIMINARY AMENDMENT**

**In the Specification**

After the specification and before the claims please insert the enclosed Sequence Listing as pages 51-81. Please renumber original pages 51-59 as 82-90, respectively.

At page 1, line 26, please delete "In." and insert therefor --In--.

At page 8, line 11, please delete "on" and insert therefor --as--.

At page 11, line 7, please delete "WO 94/115116" and insert therefore --WO 94/11516--.

At page 12, line 12, please delete "WO 94/115116" and insert therefore --WO 94/11516--.

At page 43, lines 16-19, please delete "non-polar residue, leucine, for a polar residue, histidine" and insert therefor --polar residue, histidine, for a non-polar residue, leucine--.

At page 44, line 23, after "3'", please insert --(SEQ ID NO:19)--. At line 24, after "3'", please insert --(SEQ ID NO:20)--. At line 26, after "3'", please insert --(SEQ ID NO:21)--. At line 27, after "3'", please insert --(SEQ ID NO:22)--.

At page 49, line 7, after "CAUCAUCAUCAUCTTCTTCGTAGGGTTCATCG", please insert --(SEQ ID NO:23)--. At line 8, after "CUACUACUACUATCATAGAAGAGAAAGGTTTCAG", please insert --(SEQ ID NO:24)--. At line 13, after "3'", please insert --(SEQ ID NO:25)--. At line 14, after "3'", please insert --(SEQ ID NO:26)--.

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No new matter is added by these amendments.

### **In the Claims**

Please cancel claims 10, 15, 20-25, 27, and 31 without prejudice to further prosecution.

Please amend the claims as follows:

1. (Amended) An isolated nucleic acid fragment comprising a sequence of at least about 10 nucleotides from a Brassicaceae or Helianthus delta-12 fatty acid desaturase gene having at least one mutation in a region of said desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said [gene is effective for altering fatty acid composition in Brassicaceae or Helianthus seeds] at least one mutation renders the product of said desaturase gene non-functional and wherein said sequence includes said at least one mutation.
3. (Amended) The nucleic acid fragment of claim 2, wherein said at least one mutation [comprises] in said gene introduces a non-conservative amino acid substitution in said [region] motif.
4. (Amended) An isolated nucleic acid fragment comprising a sequence of at least about 10 nucleotides from a Brassicaceae or Helianthus delta-15 fatty acid desaturase gene having at least one mutation in a region of said desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said [gene is effective for altering fatty acid composition in Brassicaceae or Helianthus seeds] at least one mutation renders the product of said desaturase gene non-functional and wherein said sequence includes said at least one mutation.
6. (Amended) The nucleic acid fragment of claim 5, wherein said at least one mutation [comprises] in said gene introduces a non-conservative amino acid substitution in said [region] motif.
7. (Amended) An isolated nucleic acid fragment encoding a polypeptide having an amino acid sequence selected from the group consisting of: [an amino acid sequence substantially identical to] SEQ ID NO:12, [an amino acid sequence substantially identical to] and SEQ ID NO:16 [and an amino acid sequence substantially identical to SEQ ID NO:18].

11. (Amended) An isolated nucleic acid fragment, wherein said nucleic acid fragment is selected from the group consisting of:

a) SEQ ID NO:11;

b) SEQ ID NO:15;

c)[ SEQ ID NO:17;

d)] an RNA [analog] comprising a nucleotide sequence of SEQ ID NO:11, wherein uracil replaces thymine;

[e]d] an RNA [analog] comprising a nucleotide sequence of SEQ ID NO:15, wherein uracil replaces thymine;

[f] an RNA analog of SEQ ID NO:17;

g]e] a nucleic acid fragment having a nucleic acid sequence complementary to a), b), c), or d)[, e), or f)]; and

[h]f] a nucleic acid fragment of a), b), c), d), or e)[, f), or g),] that is at least 10 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the mutation in the polypeptide of SEQ ID NO:12, or SEQ ID NO:16[, or SEQ ID NO:18].

12. (Amended) An isolated polypeptide having an amino acid sequence selected from the group consisting of: [an amino acid sequence substantially identical to] SEQ ID NO:12[, and [an amino acid sequence substantially identical to] SEQ ID NO:16[, and an amino acid sequence substantially identical to SEQ ID NO:18].

16. (Amended) A Brassicaceae or Helianthus plant, said plant containing first and second delta-12 fatty acid desaturase genes, each said gene having at least one mutation, wherein at least one of said mutations is in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif and wherein each said mutation renders the product of said desaturase gene non-functional, and confers an altered fatty acid composition in seeds of said plant.

In claim 18, please delete "region" and insert therefor --motif--.

26. (Amended) A method for producing a Brassicaceae or Helianthus plant line, comprising the steps of:

- a) inducing mutagenesis in cells of a starting variety of a Brassicaceae or Helianthus species;
- b) obtaining one or more progeny plants from said cells;
- c) identifying at least one of said progeny plants that contain[s] a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, and wherein said mutation renders the product of said desaturase gene non-functional; and
- d) producing said plant line from said at least one progeny plant by self- or cross-pollination, said plant line having said at least one delta-12 gene mutation and producing seeds yielding an oil having a linoleic acid content from about 1% to about 14%.

28. (Amended) The method of claim 26, further comprising the steps of:

- e) inducing mutagenesis in cells of said plant line;
- f) obtaining one or more progeny plants from said plant line cells;
- g) identifying at least one of said plant line progeny plants that contains a delta-15 fatty acid desaturase gene having at least one delta-15 gene mutation, said at least one delta-15 gene mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-15 desaturase gene non-functional;
- h) producing a second plant line from said at least one plant line progeny plant by self- or cross-pollination, said second plant line having said at least one delta-12 gene mutation and said at least one delta-15 gene mutation and producing seeds yielding an oil having a linoleic acid content from about 1% to about 14%.

30. (Amended) A method for producing a Brassicaceae plant line, comprising the steps of:

- a) inducing mutagenesis in cells of a starting variety of a Brassicaceae species;
- b) obtaining one or more progeny plants from said cells;

c) identifying at least one of said progeny plants that contains a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said desaturase gene non-functional;

d) producing a first plant line from said at least one progeny plant by self- or cross-pollination, said plant line having said at least one delta-12 gene mutation;

e) inducing mutagenesis in cells of said first plant line;

f) obtaining one or more progeny plants from said first plant line cells;

g) identifying at least one of said first plant line progeny plants that contains a second delta-12 fatty acid desaturase gene having at least one mutation, said second gene mutation in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif; and

h) producing a second plant line from said at least one plant line progeny plant by self- or cross-pollination, said second plant line having said first delta-12 gene mutation and said second delta-12 gene mutation.

Please add the following new claims.

35. The nucleic acid fragment of claim 11, wherein said nucleic acid fragment is SEQ ID NO: 11.

36. The nucleic acid fragment of claim 11, wherein said nucleic acid fragment is SEQ ID NO: 15.

37. The plant of claim 16, wherein said plant is a Brassicaceae plant.

38. The plant of claim 37, wherein said plant is a Brassica napus plant.

39. The plant of claim 37, wherein said motif comprises the sequence His-Glu-Cys-Gly-His.

40. The plant of claim 37, wherein said mutation comprises a non-conservative amino acid substitution in said motif.

41. The method of claim 26, wherein said starting variety is a Brassicaceae species.

42. The method of claim 41, wherein said identifying step comprises identifying a mutation in a His-Glu-Cys-Gly-His amino acid motif.

43. The method of claim 28, wherein said starting variety is a Brassicaceae species variety.

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44. The method of claim 43, wherein said identifying step comprises identifying a mutation in a His-Glu-Cys-Gly-His amino acid motif.

45. The method of claim 43, wherein said producing step h) comprises producing seeds yielding an oil having an a-linolenic acid content from about 0.5% to about 10%.

46. The method of claim 30, wherein said identifying step b) comprises identifying a mutation in a His-Glu-Cys-Gly-His amino acid motif.

### REMARKS

Applicants have amended claims 1, 3-4, 6-7, 16, 18, 26, 28, and 30, and cancelled claims 10, 15, 20-25, 27, and 31 without prejudice to further prosecution. Claims 35-46 are submitted herewith. Applicants respectfully request consideration and allowance of claims 1-9, 11-14, 16-19, 26, 28-30, and 32-46.

Claims 1 and 4 have been amended to recite that the mutation is in a region of the desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif. Claims 1, 4, 16, 26, and 30 have been amended to recite that the mutation renders the desaturase gene non-functional. Support for these amendments can be found, for example, at page 8, lines 26-29, and at page 10, lines 29-33.

The amendments to the specification at pages 1, 8, 11-12, 43, 44 and 49 serve to correct typographical errors and add sequence identifiers.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: January 29, 2001

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